Embryo culture and rapid propagation of *Syringa*

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Abstract: Embryo of lilacs (*Syringa* L) culture in vitro and the rapid propagation were studied. The orthogonal experiments, including the selection of basal medium, embryo age and other factors such as sugar, benzyladenine (BA), naphthalene acetic acid (NAA) and glutamine (Gln), were carried out. The results indicated that the optimal medium for embryo culture was Monnier medium supplemented with NAA (0.001 mg·L^{-1}), BA (0.1 mg·L^{-1}), sugar (50 g·L^{-1}), and Gln (400 mg·L^{-1}), with a germination rate of 91.7% at least; the optimal embryo age was 50 d; and Gln had significant effects on the germination rate of embryo. Moreover, the optimal medium for subculture was MS+BA (2 mg·L^{-1})+NAA (0.001 mg·L^{-1})+Gln (0.5 mg·L^{-1}), with the propagation coefficient of 3.6 at least.

Keywords: *Syringa*; Embryo-culture; Rapid propagation
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Introduction

Lilac *(Syringa* L*)* is a famous ornamental shrub in osmanthus *(Oleaceae)* and widely used in landscaping (Zang 1990). It is also regarded as one of the most important plants for planting around the factory and diggings because of its better tolerance to noxious gases such as HF, SO_2 etc. In order to make full use of this plant, botanists have been continuously breeding the new varieties of *Syringa* for over 300 years (Vrugtman 1994), and nearly 400 cultivars have been developed (Zang 1993). However, the embryo of crossbreeding frequently died before the seed matured, and the success rate of hybridization generally kept very low (Zang 1983). For improving the success rate, auximone and other chemicals were used during the course of the crossbreeding, but the results were not satisfied (Liu 1995). Embryo culture in vitro was a part of the biotechnologies used in the crossbreeding of plants, but only few reports about *Syringa* (Li 1992; Pikaleva 1992).

This paper studied the embryo culture in vitro and the rapid propagation of *Syringa*, determined the optimal basal medium, the correlation of the concentration of sugar source and embryo age, and the effect of other factors on the germination rate of embryo including the BA concentration, the NAA concentration, and the Gln concentration. Since the traditional breeding method could not meet the need of production, the subcultures of the seedlings of *Syringa* were also studied for improving the breeding coefficient. This study not only could meet practical and scientific needs, but also has an important significance in the cultivation and application of new variety of *Syringa*.

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Material and method

Selection of initial culture medium

The embryos of *Syringa* were inoculated initially on eight mediums as follows: MS, SH, Amend-White, Amend-N_6, Monnier, Wpm, LS, DCR, and cultured at the temperature of (25±2)°C. These mediums were supplemented with 0.6% agar. The pH value was between 5.8-6.2. The time and intensity of illumination were 14 hours and 1 000-1 200 Lux. The germination rate on each medium was investigated in 30 days (The same latter).

Correlation of sugar concentration and embryo age

The embryos of *Syringa* were inoculated after pollination, with once every 10 days from 30 to 90 d. The Monnier mediums were prepared by separately supplementing 20, 30, 40, 50, 60, 70, and 80 g·L^{-1} sugar, to each of them

Selection of the other influencing factors

Five factors were selected by the $L_{16}(4^5)$ orthogonal experiment, which may have influence on the germination rate of the embryo, and the factors and their levels were designed as follows:

A (Sugar concentration): A_1, 40 g·L^{-1}; A_2, 50 g·L^{-1}; A_3, 60 g·L^{-1}; A_4, 70 g·L^{-1};

B (Embryo age): B_1, 40 d; B_2, 50 d; B_3, 60 d; B_4, 70 d;

C (Gln concentration): C_1, 100 mg·L^{-1}; C_2, 200 mg·L^{-1}; C_3, 300 mg·L^{-1}; C_4, 400 mg·L^{-1};

D (BA concentration): D_1, 0 mg·L^{-1}; D_2, 0.001 mg·L^{-1}; D_3, 0.01 mg·L^{-1}; D_4, 0.1 mg·L^{-1};

E (NAA concentration): E_1, 0 mg·L^{-1}; E_2, 0.001 mg·L^{-1}; E_3, 0.01 mg·L^{-1}; E_4, 0.1 mg·L^{-1}.

The embryos were inoculated on the above sixteen different mediums. Observation and investigation were made in 30 days.

Selection of the subculture mediums

The $L_{16}(4^5)$ orthogonal experiment was designed as well. The seedlings of *Syringa* were transplanted onto the other sixteen different mediums and the breeding coefficients were calculated in 30 days.

Results and analysis

Effect of different mediums on the germination and formation of embryo of *Syringa*

By observing and counting, we knew that the germinating rate of embryo had obvious difference. The actual results were shown in Fig. 1.

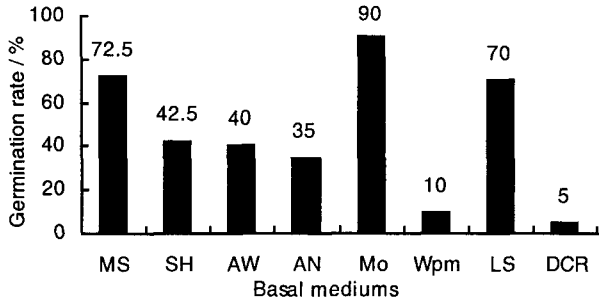

Fig. 1 Effect of different medium on the germination of embryo of *Syringa*

AW: Amend-White; AN: Amend-N_6, Mo: Monnier

The average germination rate of the embryos on the Monnier medium was the highest (90%), followed by those on the MS and LS mediums (72.5% and 70%). The seedlings grown on the Monnier medium were very vigorous and had obvious growth but those on MS and LS mediums seemed very slim and fragile. Other several kinds of mediums were not suitable for embryo culture of *Syringa* (Fig. 1).

By analyzing the components of above mediums (Tan 1991), we found that the macro-element and microelement in various mediums had distinct differences. In Monnier medium, the Ca^{2+} and all microelements were almost as twice as those in MS, and the KCL was added to increase the K^+ level and the Fe-EDTA was nearly reduced to the half. All these maybe accelerate the germination of the embryo.

Correlation of sugar concentration and embryo age

The embryo age and sugar concentration had close relation, which directly affected the germination rate of the embryo. When the embryo age was 50-60 d and the sugar concentration was 50 $g \cdot L^{-1}$ the germination rate was the highest (90%-100%). The results indicated that the bigger the embryo age was, the lower the sugar concentration it needed was (See Table 1)(Lv 1981).

Table 1. Effect of different embryo age and sugar concentrations on germination of embryo in *Syringa*

Embryo age /d	Germination rate (%) at different sugar concentration ($g \cdot L^{-1}$)						
	20	30	40	50	60	70	80
40	0	0	0	40	60	65	20
50	20	35	90	100	85	55	35
60	25	40	85	90	35	30	20
70	15	45	80	65	30	15	0
80	25	20	45	10	0	0	0
90	10	30	25	5	0	0	0

From the variance analysis (Table 2), we could see that both the embryo age and the sugar concentration had significant effect on the germination rate, especially the embryo age. From the SSR test of significance (Table 3), the same conclusions could be drawn as the above analysis. Thus it was determined that, during the course of the embryo culture, the optimal embryo age was 50-60 d and the optimal sugar concentration was 50 $g \cdot L^{-1}$.

Table 2. Variance analysis of the effect of different embryo age and sugar concentration

Variance origin	Freedom	Sum of Square error	Mean square	Mean Square rate	$F_{0.05}$	$F_{0.01}$
Embryo age	5	12842.86	2568.57	5.39**	2.53	3.7
Sugar concentration	6	9373.81	1562.30	3.28*	2.42	3.47
Error	30	14290.47	476.35			
Variance	41	36507.14				

The selection of the other influencing factors

The results of orthogonal experiment were shown in the Table 4.

According to the T value, the best combination of the five factors was $A_2B_2C_4D_4E_2$, i.e. the factor A (sugar concentration) was 50 $g \cdot L^{-1}$ (A_2); factor B (embryo age) was 50 d (B_2); factor C (Gln concentration) was 400 $mg \cdot L^{-1}$ (C_4); factor D (BA concentration) was 0.1 $mg \cdot L^{-1}$ (D_4); factor E (NAA concentration) was 0.001 $mg \cdot L^{-1}$ (E_2).

According to the R-value, we could see from Table 4 that the R-value from high to low was $R_C > R_D > R_A > R_B > R_E$. The R_C was the highest, so the factor C (Gln concentration) had significant effect on the germination rate.

The results of variance analysis were shown as Table 5.

From Table 5, we could see that the effect of these factors on the germination rate from high to low was C (Gln concentration) > D (BA concentration) >A (sugar concentration) > B (embryo age) > E (NAA concentration). It was accordant with the results of direct analysis. There were significant differences between the Gln concentration and embryo age, and Gln concentration and NAA concentration.

Thus, according to the analysis above-mentioned, the

most optimal medium for embryo culture was Monnier + sugar (50 g·L^{-1})+ Gln (400 mg·L^{-1})+BA (0.1 mg·L^{-1})+NAA (0.001 mg·L^{-1}), and the germination rate was the highest (91.7%).

Table 3. The SSR test of significance of germination of embryo with different age and different sugar concentration

Embryo age /d	Average germination rate	Difference		Sugar concentration /g·L^{-1}	Average germination rate	Difference	
		$F_{0.05}$	$F_{0.01}$			$F_{0.05}$	$F_{0.01}$
50	60	a	A	50	54.17	a	A
60	46.43	b	AB	40	51.67	a	AB
70	35.71	bc	BC	60	35	b	BC
40	26.42	cd	CD	30	28.33	bc	CD
80	14.29	d	D	70	27.50	bc	CD
90	10	e	D	20	15.83	c	D
				80	12.5	c	D

1) The same capital letter in the fourth and eighth column indicates that the difference of fresh pollen vitality between them are not extremely significant at the 0.01 level; The different capital letter in the fourth and eighth column indicates that the difference between them is extremely significant at the 0.01 levels; 2) The small letter in the third and seventh column indicates the difference at the 0.05 levels

Table 4. Result of orthogonal tests of embryo culture in *Syringa*

Number	A 1	B 2	C 3	D 4	E 5	Germinating rate	Number	A 1	B 2	C 3	D 4	E 5	Germinating rate
Z_1	1	1	1	1	1	12.5	Z_9	3	1	3	4	2	83.3
Z_2	1	2	2	2	2	20.8	Z_{10}	3	2	4	3	1	75
Z_3	1	3	3	3	3	41.6	Z_{11}	3	3	1	2	4	16.9
Z_4	1	4	4	4	4	75.7	Z_{12}	3	4	2	1	3	18.3
Z_5	2	1	2	3	4	33.2	Z_{13}	4	1	4	2	3	58.1
Z_6	2	2	1	4	3	87.5	Z_{14}	4	2	3	1	4	37.5
Z_7	2	3	4	1	2	91.7	Z_{15}	4	3	2	4	1	25
Z_8	2	4	3	2	1	49.9	Z_{16}	4	4	1	3	2	16.6
T_1	150.6	187.1	133.5	160	162.4		X_1	37.65	46.78	33.38	40.00	40.60	
T_2	262.3	220.8	97.3	145.7	212.4		X_2	65.58	55.20	24.33	36.43	53.10	
T_3	193.5	175.2	212.3	166.4	205.5	T=743.6	X_3	48.38	43.80	53.08	41.60	51.38	
T_4	137.2	160.5	300.5	271.5	163.3		X_4	34.30	40.13	75.13	67.88	40.83	
R	31.28	15.07	50.8	31.45	12.51								

Table 5. Variance analysis of orthogonal test result from Table 4

Variance origin	Freedom	Sum of square error	Mean square	Mean square rate	$F_{0.05}$	$F_{0.01}$
C (Gln concentration)	3	6106.46	2035.49	12.34* 11.36* 2.57 2.44	9.28	29.5
D (BA concentration	3	2498.62	832.87	5.05 4.65 1.05		
A (Sugar concentration)	3	2378.13	792.71	4.81 4.43		
B (Embryo age)	3	537.36	179.12	1.09		
E (NAA concentration)	3	494.78	164.93			
Variance	15	12015.35				

Selection of the subculture medium

The results of orthogonal experiment in the phase of subculture were shown in Table 6.

According to T value, the propagation coefficient was the highest when the medium was MS+BA (2 mg·L^{-1})+NAA (0.1 mg·L^{-1})+IBA (0.5 mg·L^{-1})+Gln (100 mg·L^{-1}).

According to R-value, the influence of BA concentration was the most significant, and the second was basal medium.

The variance analysis of orthogonal test results was shown in Table 7. The effect on the propagation coefficient from high to low was BA concentration > basal medium > IBA concentration > Gln concentration > NAA concentration. It was accordant with the direct analysis.

Thus during the subculture of seedlings of *Syringa*, the most optimal medium was MS +BA (2 mg·L^{-1})+NAA (0.1 mg·L^{-1})+IBA (0.5 mg·L^{-1})+Gln (100 mg·L^{-1}), and the propagation coefficient could reach 3.6 at least.

Table 6. Orthogonal tests about proliferation culture of *Syringa*

Number	Medium	Concentrations				Propagation coefficient
		BA /mg·L^{-1}	NAA /mg·L^{-1}	IBA /mg·L^{-1}	Gln /mg·L^{-1}	
Z_1	Monnier	1	0.01	0	100	2.5
Z_2	Monnier	2	0.02	0.5	200	3.6
Z_3	Monnier	3	0.03	1	300	1.7
Z_4	Monnier	4	0.04	2	400	0.4
Z_5	MS	1	0.02	1	400	3.1
Z_6	MS	2	0.01	2	300	3.4
Z_7	MS	3	0.04	0	200	3.1
Z_8	MS	4	0.03	0.5	100	2.9
Z_9	LS	1	0.03	2	200	1.2
Z_{10}	LS	2	0.04	1	100	3
Z_{11}	LS	3	0.01	0.5	400	2.3
Z_{12}	LS	4	0.02	0	300	0.2
Z_{13}	SH	1	0.04	0.5	300	2.4
Z_{14}	SH	2	0.03	0	400	1.8
Z_{15}	SH	3	0.02	2	100	0.9
Z_{16}	SH	4	0.01	1	200	0.2
T_1	8.2	9.2	8.4	7.6	9.3	
T_2	12.5	11.8	7.8	11.2	8.1	
T_3	6.7	8	7.7	8	7.7	T=32.70
T_4	5.3	3.7	8.9	5.9	7.6	
R	1.8	2.02	0.3	1.32	0.43	
X_1	2.05	2.30	2.10	1.90	2.33	
X_2	3.13	2.95	1.95	2.80	2.03	
X_3	1.68	2.00	1.93	2.00	1.93	
X_4	1.33	0.93	2.23	1.48	1.90	

Conclusion and discussion

The optimal basal medium for embryo culture in vitro was Monnier, and the second was MS or LS. It indicated that the embryo of *Syringa* needs abundant macro-element and microelement, especially the Ca^{2+} and K^+ at high level.

The optimal sugar concentration for embryo culture in vitro was 50 g·L^{-1}. At this concentration, the medium could offer enough nutrition and the high osmotic pressure for embryo.

The optimal embryo age for embryo culture in vitro is 50-60 d. At this time, the cotyledon in ovule began to form or organ began to differentiate, so the embryo was easy to germinate and the seedling was easy to form.

The Gln concentration had significant effect on embryo culture, the optimal medium was Monnier + sugar (50 g·L^{-1})+BA (0.1 mg·L^{-1})+NAA (0.001 mg·L^{-1})+Gln (400 mg·L^{-1}), with the germination rate of 91.7%.

The BA concentration had significant effect on multiplication, the optimal medium was MS + BA (2 mg·L^{-1})+NAA (0.01 mg·L^{-1})+ IBA (0.5 mg·L^{-1}) +Gln (100 mg·L^{-1}), with the propagation coefficient of over 3.6.

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Table 7. Variance analysis of orthogonal test results

Variance origin	Freedom	Sum of square error	Mean square	Mean square rate	$F_{0.05}$	$F_{0.01}$
BA concentration	3	8.56	2.85	35.67** 8.61* 2.33 1.17	9.28	29.5
Basal medium	3	7.29	2.43	30.38** 15.85*1.99		
NAA concentration	3	3.67	1.22	15.29* 7.98		
Gln concentration	3	0.46	0.15	1.92		
NAA concentration	3	0.24	0.08			
Total difference	15	20.22				